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TITLE: Investigation of Lobular Carcinoma In Situ, Using Molecular Genetic Techniques, for the Involvement of Novel Genes

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13. ABSTRACT (Maximum 200 Words)

Atypical lobular hyperplasia (ALH) and lobular carcinoma *in situ* (LCIS), i.e. lobular neoplasia, are lesions of significance in terms of implication of risk to the patient in the development of invasive carcinoma. A strong correlation between the lobular histological type and inactivation of E-cadherin, a protein involved in cell adhesion, has been reported. As well, mutations in the E-cadherin gene have been reported in invasive lobular carcinoma (ILC) and LCIS with adjacent ILC. The purpose of our study is to investigate lobular neoplastic lesions, lacking any adjacent invasive carcinoma, for alterations in and expression of known and novel genes/proteins with the goal of characterizing a molecular genetic profile for lobular neoplasia. We have accrued 23 cases of which there are 14 ALH lesions and 14 LCIS lesions. All cases able to be evaluated are negative for E-cadherin and beta-catenin protein expression by immunohistochemistry. The mutation analysis has been completed for thirteen lesions and to date alterations in the E-cadherin gene have only been characterized in LCIS. All ALH lesions screened to date show inactivation of E-cadherin but harbor no alterations. Studies are in progress to evaluate loss of heterozygosity at chromosome 16q, E-cadherin promoter methylation, and p120-catenin protein expression. The completion of these analyses will provide insight into the molecular genetic profile for lobular neoplasia.

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Introduction

Tumor development from an early lesion through to invasive disease is not a clearly defined progression in the breast. A continuum can be hypothesized for breast lesions of the lobular histological type, from hyperplasia through *in situ* carcinoma to invasive breast carcinoma (IBC). However, discovering the specific molecular genetic events that mark the transition from an early lobular lesion to an invasive tumor is necessary to both support and subsequently understand this potential lobular progression.

Lobular neoplasia is a histological classification that includes atypical lobular hyperplasia (ALH) and lobular carcinoma *in situ* (LCIS).[1] Both ALH and LCIS are found incidentally during breast tissue biopsy due to their inability to be detected by palpation or mammography. Histologically there is a proliferative gradation from ALH to LCIS that is also reflected in the relative risk to the patient in the development of IBC. A finding of ALH imply a four to five fold increased risk of subsequent carcinoma in either breast, and a finding of LCIS implies an eight to ten fold increased risk to the patient.[2-4]

A strong correlation between the lobular histological type and inactivation of the cell adhesion protein epithelial(E)-cadherin has been reported.[5-11] As well, mutations in the E-cadherin gene have been found in invasive lobular carcinoma (ILC) and LCIS with adjacent ILC.[5-11] Our studies look to investigate both ALH and LCIS lesions, lacking any adjacent invasive carcinoma, for alterations in and expression of known and novel genes/proteins with the goal of characterizing a molecular genetic profile for lobular neoplasia.

Key Research Accomplishments

Since the inception of the study, the type of lesion being investigated has been expanded to include both types of lobular neoplastic lesions, i.e. ALH and LCIS. Tasks proposed in the original Statement of Work that looked solely to investigate LCIS lesions will now also be carried out on the accrued ALH lesions.

Task 1: Tissue Accrual

We have expanded the criteria for cases included in the study to include ALH, LCIS, or both ALH and LCIS. The original collection has been re-reviewed to identify all the lobular neoplastic lesions in each case. The number of cases in the study is currently at 23, including 14 ALH lesions and 14 LCIS lesions (with five cases housing both ALH and LCIS). All cases in the collection are formalin-fixed, paraffin-embedded breast tumor blocks containing lobular neoplastic lesions lacking adjacent invasive carcinoma. The accrual of cases remains ongoing. See appendix 1 for the detailed description of the current collection.

Task 2: Completion of the Analysis of E-cadherin in LCIS

The analysis has been completed for alterations in the E-cadherin gene in the original cases. As new cases are added to the collection, mutation analysis is being completed. A list of alterations characterized to date has been appended (appendix 1).

Task 3: Analysis of LCIS by Chromosomal Comparative Genome Hybridization (CGH)

The analysis of ALH and LCIS lesions by chromosomal CGH has not yet begun.

Task 4: Analysis of LCIS by CGH Microarray

In our laboratory, a CGH microarray protocol is currently being optimized and evaluated for accuracy and efficiency. Until this technique has been validated, the analysis of our lobular neoplastic lesions by CGH microarray will not commence.

Task 5: Analysis of LCIS by Immunohistochemistry (IHC)

As new cases are accrued, staining for protein expression by IHC is carried out. To date all cases have been stained for E-cadherin. Subsequently, staining for beta-catenin by IHC has also been optimized and completed on all cases currently in the collection. A scoring system has been developed in collaboration with Dr. Frances O'Malley for both E-cadherin and beta-catenin. A complete lack of E-cadherin and beta-catenin protein expression has been found in all cases of ALH and LCIS evaluated by IHC (appendix 1). Each lesion contains an adjacent internal positive control. As well, we have included a case of DCIS as a positive control for all IHC experiments. Currently, optimization of the p120-catenin antibody for IHC is in progress.

Additional work: Evaluation of methods of inactivation of E-cadherin in lobular neoplasia.

The results from our studies to date have suggested that in lobular neoplastic lesions E-cadherin may be inactivated by means other than the presence of mutation(s). To address this, other methods of E-cadherin inactivation, namely loss of heterozygosity (LOH) and E-cadherin promoter methylation, are being evaluated.

LOH analysis of chromosome 16q, which houses the E-cadherin gene, is being determined with the use of five microsatellite markers. The markers are located at 16q22.1/16q22.2 and fall upstream (D16S503, D16S496), downstream (D16S752, D16S3095) and within the E-cadherin gene (D16S421). This analysis has been completed for the original cases, and as new cases are added to the collection the LOH status for each lesion will be determined. The LOH results to date have been compiled in appendix 1.

The assessment of E-cadherin promoter methylation has also been proposed to be carried out on our lobular neoplastic lesions. Optimization of a methylation specific PCR protocol is in progress.

Reportable Outcomes

1. "E-cadherin alterations in lobular neoplasia". Manuscript is in preparation.
2. "Characterization of a molecular genetic profile for lobular neoplasia". Proceeding of the American Association of Cancer Research (1st Edition), volume 44, 2003.
3. "Characterization of a molecular genetic profile for lobular neoplasia". Department of Laboratory Medicine and Pathobiology Graduate Student Research Day, University of Toronto, March 2003.
4. "Analysis of E-cadherin in Lobular Carcinoma *in situ*". Proceedings of the American Association of Cancer Research, volume 43, 2002.
5. "Profiling Lobular Neoplasia". Center for Cancer Genetics Seminar Series, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, March 2002.
6. "Analysis of E-cadherin in Lobular Neoplasia". Department of Laboratory Medicine and Pathobiology Graduate Student Research Day, University of Toronto, March 2002.
7. "Analysis of E-cadherin in Lobular Neoplasia". Divisional Seminar Series, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, February 2002.

Conclusions

Cases lacking both expression of E-cadherin and gene alterations suggest that another mechanism, involved at the stage of hyperplasia, is causing the lack of protein expression. In light of these findings, studies are in progress to evaluate other mechanisms of gene silencing i.e. LOH and promoter methylation, as well as the expression status of proteins associated with E-cadherin. The completion of these analyses will provide insight into the molecular genetic profile for lobular neoplasia.

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Appendix 1: Summary of results from Case Accrual, E-cadherin Mutation Analysis, Immunohistochemistry, and LOH Analysis.

	Case Accrual		Mutation Analysis				Immunohistochemistry				LOH
Case Code	Original Study	Pathology	Alteration	Exon	bp	Effect	E-cadherin IHC	Internal Control	Beta-catenin IHC	Internal Control	LOH
A1	Yes	ALH	none				-	+	-	+	No
A2(L1)	Yes	ALH	none				-	+	-	+	No
A3(L3)		ALH	IP				-	+	-	+	IP
A4(L5)		ALH	IP				-	+	-	+	IP
A5	Yes	ALH	none				-	+	-	+	No
A6	Yes	ALH	none				N/A	N/A	N/A	N/A	N/A
A7	Yes	ALH	IP				-	+	N/A	N/A	No
A8		ALH	IP				-	+	-	+	IP
A9		ALH	none				-	+	-	+	IP
A10		ALH	IP				-	+	-	+	IP
A11(L12)		ALH	IP				-	+	-	+	IP
A12		ALH	IP				-	+	-	+	IP
A13		ALH	IP				-	+	-	+	IP
A14(L14)		ALH	IP				-	+	-	+	IP
L1(A2)	Yes	LCIS	GCC - ACC CAC - CGC	7 3	856 362	Ala → Thr His → Arg	-	+	-	+	Yes
L2	Yes	LCIS	CAT - TAT	3	274	His → Tyr	-	+	-	+	No
L3(A3)	Yes	LCIS	GCC - ACC	13	2125	Ala → Thr	-	+	-	+	MSI
L4	Yes	LCIS	11bp deletion	10	1417-1427	Frameshift, Stop	-	+	-	+	No
L5(A4)	Yes	LCIS	GTG - ATG	10	1459	Val → Met	-	+	-	+	No
L6	Yes	LCIS	AGC - AAC	11	1676	Ser → Asn	-	+	-	+	MSI
L7	Yes	LCIS	GGT - GAT	3	185	Gly → Asp	-	+	-	+	Yes
L8	Yes	LCIS	2 bp deletion	9	1309-1310	Frameshift, Stop	-	+	N/A	N/A	No
L9		LCIS	IP				-	+	-	+	N/A
L10		LCIS	IP				-	+	-	+	No
L11		LCIS	IP				N/A	N/A	N/A	N/A	No
L12(A11)		LCIS	IP				-	+	-	+	IP
L13		LCIS	IP				-	+	-	+	IP
L14(A14)		LCIS	IP				-	+	-	+	IP
Positive	Yes	DCIS	none				+	+	+	+	N/A

(ALH) atypical lobular hyperplasia; (LCIS) lobular carcinoma *in situ*; (DCIS) ductal carcinoma *in situ*; (bp) base pair; (-) negative protein expression; (+) positive protein expression; (N/A) tissue not available for experiment; (IP) in progress.